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THE EFFECT OF SEVERE BURNS AND SOME PROTEIN-PRECIPITANTS ON SKIN-HISTAMINE IN CATS

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In previous experiments (Dekanski, 1945) it was found that moderate cutaneous burns in mice caused the formation of histamine, mainly in the skin. The total amount of histamine in the mouse was almost doubled in 10 min. and reached its maximum concentration within 1 hr. or less. If the mouse survived, the excess histamine was excreted in the urine during the next 48 hr.

These results confirm those of Kisima (1938) and Lambert & Rosenthal (1943), but not those of Harris (1927). Harris found that when the skin of an anaesthetized cat was burned with a hot flat-iron there was oedema, but no change in the histamine concentration in the burnt area of skin, during the first hour. After this time, he found gradual absorption of the histamine originally present, along with the oedema fluid. It was decided to investigate the discrepancy between the results obtained in this laboratory and those of Harris by performing experiments in which the histamine content of skin in cats was followed after moderate and severe burns.

Experiments were also carried out to examine the effect of various substances, which have been used in the treatment of burns, on the histamine content of normal and moderately burnt skin.

Methods

The cats used were prepared on the previous day by shaving each flank. They were anaesthetized with sodium pentobarbitone ('Veterinary Nembutal', Abbott Laboratories Ltd.), given intravenously after induction with ether. Three or four areas were then outlined on each flank by means of a rubber stamp, so as to obtain six or eight patches of skin of equal surface area. Each of the outlined areas, except one or two controls in each experiment, was then burned at 60° C. or 80° C. for 60 sec. by means of the apparatus described by Leach, Peters & Rossiter (1943), or by means of a hot metal rod at 140° C. for 15 sec.

In the second series of experiments, burning was done at 60° C. for 60 sec. only, and most of the burnt areas, in addition to some unburnt areas, were treated with chemical solutions. The substances examined were 20% tannic acid, 10% silver nitrate, 2% gentian violet, 1% formalin (i.e. 1% solution of 40% formaldehyde) and 2% 'Mercurochrome', and were applied by a single, unrepeated painting, 10 min. after burning. One or two 'burnt controls' and 'unburnt controls' were left untreated.

In all these experiments after different periods, the patches of skin were removed, weighed, extracted with trichloroacetic acid (Barsoum & Gaddum, 1935), and boiled for 90 min. in conc. HCl (Code, 1937). The rest of the procedure was as originally described.

The effect in vitro of the substances on histamine itself and on the biological assay was tested by adding 150 μg. histamine phosphate to 1 c.c. of each solution and then putting the solution through the treatment with trichloroacetic acid, etc.

Estimation and identification of histamine was carried out by testing the extracts on a strip of guinea-pig's ileum suspended in 2 c.c. of Tyrode's solution containing atropine (0·1 μg./c.c.), and by intravenous injection into atropinized cats, anaesthetized as described above. The effects in all cases were compared with those obtained with standard solutions of histamine phosphate and the concentrations of histamine present were calculated in terms of histamine base. Blood-pressure readings often gave lower estimates of the histamine content than those obtained with the guinea-pig's gut. The discrepancy was never more than about 50% and usually much less and may have been due to experimental error. The results of all tests were in general agreement, and there can be little doubt that histamine was the main active principle.

Since burning alters the size and weight of the skin, results are calculated on the basis of the areas originally mapped out for treatment.

Results

Comparison of moderate and severe burns. The individual results of these experiments are given in Table 1, from the averaged results of which Fig. 1 has been constructed. The average of the weight and diameter of the outlined areas are shown graphically in Fig. 2 in which each point, except for 140° C., represents the mean of five observations.

Burning at 60° C. was found to cause an intense congestion of the skin, and very intense oedema of the subcutaneous tissues within 1 hr. The extractable histamine equivalent in the skin rose to 22·6 from the average control amount of about 10·5 μg. per outlined area. A marked increase in histamine content was observed after 10 min., the maximum value being reached within 3 hr. The histamine equivalent was still high after 5 hr.

Burning at 80° C. was found to cause a contraction and thickening of the skin and moderate congestion and oedema. The extractable skin histamine dropped from about 10·5 to 9 μg. per burned area within 1 hr., and to 4·3 μg. after 5 hr.

Burning at 140° C. for 15 sec. caused the skin to become more thickened and contracted and to appear coagulated. The subcutaneous tissues initially showed little oedema, but this became evident in the surrounding area after an interval of 30 min. or more. The histamine equivalent dropped to 9 μg. per burned area within 1 hr., and to 2·6 μg. after 5 hr.

Effect of chemicals on normal and burnt skin. The individual results of these experiments are given in Table 2, from the averaged results of which Fig. 3 has been constructed. The average of the weights is shown graphically in Fig. 2 in which each point, except for 'Mercurochrome', represents the mean of five observations. The surface area in all cases was the same as in untreated burns caused by 60° C. for 60 sec.

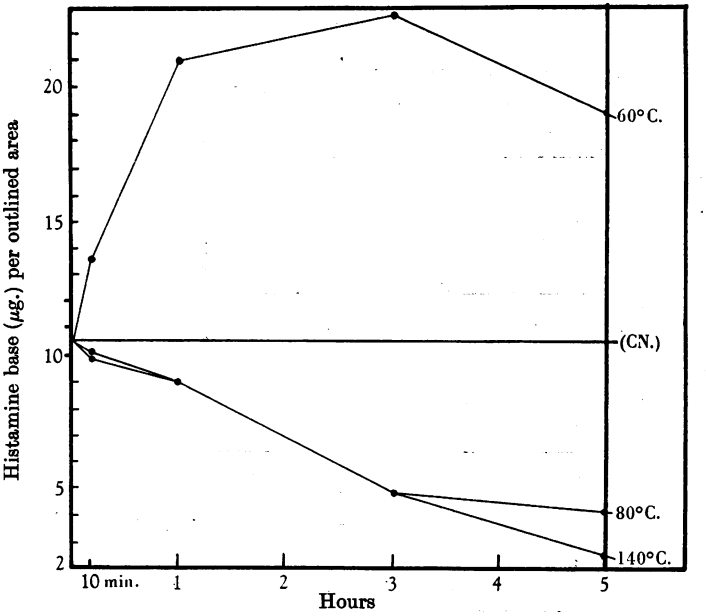

Fig. 1. Histamine equivalent at different times after burning. CN value for normal skin.

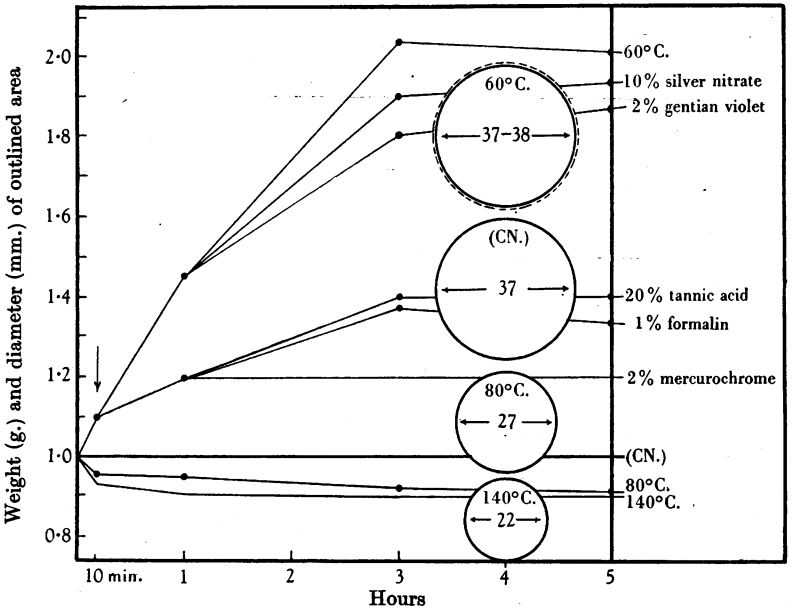

Fig. 2. Effect of burning and treatment on weight (graph) and area (circles) of cats' skin. Burns at zero time; treatment at 10 min. Burning at 60° C. increases the weight but not the area compared with unburnt controls (CN). The surface area in all cases treated was the same as in untreated burns caused by 60° C. Treatment with tannic acid, formalin and 'Mercurochrome' inhibits increase of weight. Silver nitrate and gentian violet were comparatively ineffective. Burning at 80° or 140° C. decreases the area definitely and the weight slightly.

TABLE 1. Individual results. Histamine equivalents (μg.) per area of control skin (CN) and burnt skin at 60° C. and 80° C. for 60 sec. and 140° C. for 15 sec. Extracts prepared chemically

Time after burning	60° C. I	60° C. II	80° C. I	80° C. II	140° C. II	Mean (CN)
1 min.	10·0	10·6	10·6	11·0		
	9·6	10·5	10·0	11·3		
	9·0	10·6	8·3	10·6		
	9·3	11·6	8·7	11·3	11·8	
Mean	10·1		10·2			
(CN)	*10·0*	*10·6*	*10·6*	*10·9*	—	*10·5*
10 min.	11·5	13·7	10·0	11·0		
	11·5	17·0	10·7	11·3		
	11·6	14·8	8·0	10·6		
	10·8	16·1	—	8·0	10·2	
Mean	13·4		9·9			
(CN)	*9·0*	*10·6*	*10·6*	*10·6*		*10·2*
1 hr.	20·0	20·0	—	10·5		
	17·0	23·4	10·6	10·7		
	15·6	25·3	—	8·0		
	18·0	27·9	7·0	7·9	9·0	
Mean	20·9		9·0			
(CN)	*9·4*	*10·6*	*10·7*	*10·9*	—	*10·4*
3 hr.	21·5	28·4	—	5·3		
	—	21·7	—	4·5		
	17·3	25·9	5·6	3·8		
	17·7	25·9	—	5·2	4·9	
Mean	22·6		4·9			
(CN)	—	*11·2*	—	*11·2*	—	*11·2*
5 hr.	17·2	17·2	—	3·2		
	14·5	20·2	—	3·4		
	17·4	28·7	5·5	4·5		
	15·7	20·4	—	4·8	2·6	
Mean	18·9		4·3			
(CN)	—	*11·4*	—	*11·0*	—	*11·2*

I = Cat's blood-pressure test.
II = Guinea-pig's ileum test.
(CN) = Control unburnt.

The 20 % solution of tannic acid caused diminution of congestion and oedema in the burnt skin and a significant fall in the extractable histamine equivalent to 5·8 μg. per outlined area within 3 hr., as compared with 22 μg. in burnt controls at this time and with about 10·5 μg. in normal skin. The 10 % silver nitrate did not stop the development of oedema in burnt skin. An increase in histamine was observed after 1 hr., reaching a maximum value of 24·8 μg. per outlined area within 5 hr., an even higher value than in untreated burns. After treatment with 2 % gentian violet, 1 % formalin and 2 % 'Mercurochrome' the extractable skin histamine dropped to 11·1, 9·2 and 11·7 μg. per outlined area, approximately the figures for normal skin. After treatment with gentian violet, however, there was still intense oedema. Formalin appeared to cause pain

when applied to burnt skin. The results with 'Mercurochrome' are uncertain and incomplete, as it interfered with the biological test used in the experiments.

Similar results were obtained for the histamine equivalent of control unburnt skin similarly treated with each substance. Tannic acid decreased the histamine value of normal skin, while silver nitrate increased it to about the same extent as in burnt skin, as can be seen from Fig. 3. The other substances caused no distinct change.

TABLE 2. Individual results. Histamine equivalents (μg.) per area of control skin (CB), (CT), (CN), and burnt skin at 60° C. for 60 sec. treated with the astringent solutions and 'Mercurochrome'. Extracts prepared chemically

Time after burning or treatment	20% tannic acid		10% silver nitrate		2% gentian violet		1% formalin		2% mercurochrome		Mean control
	I	II	I	II	I	II	I	II	I	II	
1 hr.	4·1	6·6	14·1	23·2	8·3	11·2	6·0	11·4			
	5·7	4·5	20·7	33·1	—	9·7	7·1	9·0			
	—	7·7	—	24·8	10·3	11·7	10·1	—			
	4·0	7·2	20·7	23·1	10·3	10·3	8·2	9·6			
	3·8	6·3	20·4	27·1	10·2	12·5	9·0	10·7	12·1	11·4	
Mean	5·5		23·0		10·5		9·0		11·7		
3 hr.	4·6	7·3	14·8	28·7	10·8	10·5	6·0	11·7			
	5·7	5·0	20·7	34·4	11·8	11·9	7·2	9·1			
	—	7·8	—	25·1	10·3	10·3	10·2	—			
	4·1	7·4	21·1	23·8	10·2	12·4	8·2	9·6			
	3·9	6·5	20·7	28·5	—	—	9·0	10·0	12·0	11·4	
Mean	5·8		24·2		11·0		9·0		11·7		
(CB)	*20·0*	*20·0*	*21·5*	*28·4*	*19·6*	*26·0*	*22·3*	*18·2*	—	—	*22·0*
5 hr.	4·6	7·4	17·3	28·5	10·8	10·2	6·8	12·0			
	5·8	5·4	21·4	34·2	12·0	12·0	7·4	9·1			
	—	7·7	—	25·6	10·4	10·4	10·1	—			
	4·1	6·5	17·0	23·4	10·4	12·7	8·0	9·5			
	3·9	5·5	22·9	33·0	—	—	9·0	10·9	12·1	11·4	
Mean	5·6		24·8		11·1		9·2		11·7		
(CB)	*17·2*	*17·2*	*17·4*	*28·7*	*24·8*	*26·4*	*14·6*	*16·0*	—	—	*20·0*
(CT)	*3·4*	*3·2*	*20·1*	*20·1*	*10·0*	*10·0*	*10·2*	*10·7*	*10·0*	*9·5*	
(CN)	*10·0*	*10·8*	*10·0*	*10·9*	—	*10·0*	*10·0*	*10·0*	—	*10·0*	*10·2*

I = Cat's blood-pressure test.
II = Guinea-pig's ileum test.
(CB) = Control burnt only.
(CT) = Control unburnt, but treated.
(CN) = Control neither burned, nor treated.

Experiments in vitro. The results in vitro (six results in each case) showed 100% recovery of histamine added to tannic acid and to gentian violet, and 98% of the histamine added to formalin. Kendall (1927) discovered that histamine is inactivated by formalin in vitro with the formation of a condensation product of histamine and formaldehyde. Best & McHenry (1931) found that formaldehyde produces an immediate effect on a solution of histamine, causing a loss of approximately 50% of its pharmacological acitivity, followed

by a slow secondary reaction in which the balance of the activity is lost if the histamine-formaldehyde solution is kept at room temperature aseptically for 3 weeks. The histamine activity lost through the formation of a condensation product is, however, almost completely restored by boiling the mixture with hydrochloric acid. Hence formalin does not actually destroy the histamine, except after a long time, and this probably accounts for the almost quantitative recovery obtained by the present procedure.

Histamine added to silver nitrate solution could not be recovered by the extraction process.

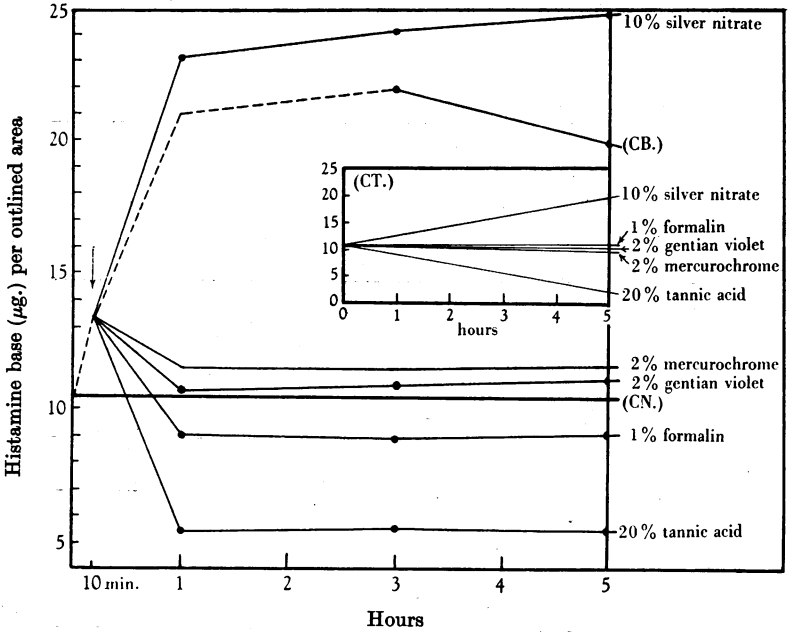

Fig. 3. Histamine equivalent at different times after treatment. Burns at zero time; treatment at 10 min. Tannic acid decreases extractable histamine equivalent in burnt skin, while silver nitrate increases it compared with values of normal skin (CN) and untreated burnt controls (CB). With other substances the values were approximately the same as in normal skin. The small inserted figure (CT) shows similar results with unburnt skin treated with each substance.

These controls suggest that the results with tannic acid, gentian violet and formalin were reliable. The results with silver nitrate render the in vivo experiments difficult to interpret. It seems that the figures obtained should be regarded only as minimal. However, the presence of protein in the animal experiments probably greatly retarded destruction of histamine by silver nitrate.

With a solution of 'Mercurochrome', although all added histamine appeared to be recovered, the compound affected both the guinea-pig's ileum and the

anaesthetized cat's blood-pressure, so that a few minutes after the first test, the preparation became abnormal in response to histamine. Thus proper assays were impossible.

Discussion

The application of heat and of various chemicals has marked effects on the amount of histamine which can be extracted from skin with trichloroacetic acid. In the experiments with mice (Dekanski, 1945) such changes were shown not to be due to the transference of histamine from one part of the body to another, but to some physical or chemical change in the skin itself. It is probable that the changes described here are also not due to the redistribution of histamine.

It is convenient to speak of the formation or destruction of histamine, but it should be made clear that this does not necessarily imply a chemical change. The extractable histamine in the skin appears to be in equilibrium with an unknown complex of some kind. This complex may consist of a chemical system which is capable of forming and destroying the molecule, or of some substance which combines with histamine or adsorbs it and renders it inert, or insoluble in trichloroacetic acid. In the experiments with mice, similar results were obtained when the histamine was extracted by electrodialysis, so that the hypothetical insoluble substance would have to be one which was not carried through cellophane to the cathode.

If these considerations are kept in mind, the histamine in extracts can be taken as a measure of the histamine content of the skin. Possible precursors, metabolic products, and inert or insoluble compounds are not included in this term, even though they may conceivably be formed merely by the adsorption of histamine by some other substance. There is no evidence that the 'conjugated histamine' described by Anrep, Ayadi, Barsoum, Smith & Talaat (1944) is present in skin. If it were, it would be hydrolyzed during the preparation of extracts and included with the free histamine in the estimates.

The first series of experiments show that the effect of burning on the histamine content of skin depends on the temperature applied. After burning at 60° C. histamine was formed in the skin. This confirms earlier results with mice. After burning at 80° C. or 140° C. no histamine was formed, but the histamine originally present disappeared over a period of several hours. This is in agreement with results of Harris (1927). In view of these facts it seems that the formation of histamine depends on the activity of irritated living cells. Dead cells form no histamine, but on the contrary slowly lose their preformed histamine. This may be destroyed, but is probably absorbed into the general circulation.

The effect of tannic acid on the histamine content of both normal and moderately burnt skin is interesting. It seems that the powerful anti-histamine action of this substance depends upon its ability to penetrate deeply into both

the normal and burnt epidermis and even into the corium. The moderately burnt skin treated with tannic acid showed less oedema. Tannic acid or tannates in excess may be absorbed in sufficient amount to damage the liver (Cameron, Milton & Allen, 1943). This may be less dangerous when treatment is applied in a single or carefully repeated spray or by swabbing in the very early stage of injury, without scraping away epidermis.

On the other hand, silver nitrate seems to have an irritating action, encouraging oedema and the formation of histamine even in unburnt skin. There is no experimental indication for silver nitrate treatment in cutaneous burns.

The effect of gentian violet is interesting, because the histamine content of the moderately burnt skin dropped to almost normal, but the oedema persisted. It seems likely that some factor or factors other than histamine were responsible for the oedema and escaped the action of gentian violet or, alternatively, that the penetration of this dye into the skin was insufficient to prevent oedema. It was observed that formalin counteracted the increase of histamine and the appearance of oedema following burns. It seems to have relatively high penetrating power but is painful if applied to burned skin. The results obtained with 'Mercurochrome' are uncertain and cannot usefully be discussed, although it has a remarkable ability to prevent oedema.

Summary

1. Small areas of cat's skin were burned under anaesthesia and then extracted with trichloroacetic acid. After moderate burns (60° C.) the histamine equivalent increased in the burned area. After severe burns (80° C. and 140° C.) the histamine originally present slowly disappeared.

2. Tannic acid prevented the development of oedema and diminished the extractable histamine of both burned and normal skin.

3. Silver nitrate increased the extractable histamine even in unburned skin, and did not prevent oedema.

4. Gentian violet prevented the increase of histamine but not the development of oedema.

5. Formalin prevented both the increase of histamine and the development of oedema.

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